

Biochemical factors contributing to tomato fruit sugar content: a review

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Abstract — Introduction. Consumers and processors value tomatoes with high fruit sugar content; however, most breeding and cultural practices negatively impact this trait. Wild tomato species can accumulate two- to three-fold more fruit sugar than cultivars and are proving to be valuable both as a source of high-sugar *loci* to broaden the genetic base of currently produced cultivars, and as research material to understand this trait. **Synthesis.** While cutting-edge genomic approaches have taught us much about fruit phenotypes, it is still important to assess fruit enzyme activities and metabolic fluxes in lines with contrasting fruit sugar accumulation. These metabolic functions are closest to the ripe fruit sugar trait. In this review, we focus our attention on the biochemical pathways, especially starch biosynthesis, that may influence tomato fruit sugars. We try where possible to put this information into a physiological context because together they influence yield. We compare and contrast sugar metabolism in cultivars and wild tomato species and identify factors that may influence differences in their fruit size. **Conclusion.** Although difficult, we show that it is possible to develop fruit with high horticultural yield and use the breeding line ‘Solara’ as an example. In addition, we suggest avenues of further investigation to understand the regulation and control of fruit carbohydrate content.

USA / *Solanum lycopersicum* / fruits / sugars / carbohydrate metabolism / carbohydrate content

Facteurs biochimiques contribuant à la teneur en sucre des fruits de tomate : une revue.

Résumé — Introduction. Les consommateurs et les industriels apprécient les tomates avec un fort taux en sucres, mais la plupart des pratiques culturales et d’amélioration ont un impact négatif sur ce caractère. Les espèces de tomate sauvage peuvent accumuler 2 ou 3 fois plus de sucres dans le fruit que des cultivars et elles s’avèrent précieuses à la fois comme une source de *loci* à haute teneur en sucres pour élargir la base génétique des cultivars actuellement produits, et comme matériel de recherche pour comprendre ce caractère. **Synthèse.** Alors que les approches génomiques de pointe nous ont appris beaucoup sur le phénotype des fruits, il reste important d’évaluer l’activité des enzymes de fruits et les flux métaboliques dans des lignées présentant des situations contrastées d’accumulation de sucres dans les fruits. Ces fonctions métaboliques sont les plus proches du caractère de teneur en sucres dans le fruit mûr. Dans cette synthèse, nous nous sommes focalisés sur les voies biochimiques, en particulier sur la biosynthèse de l’amidon qui peut influencer les sucres dans le fruit des tomates. Nous essayons autant que possible de mettre cette information dans un contexte physiologique car, ensemble, ils influencent le rendement. Nous comparons et mettons en contraste le métabolisme des sucres dans les cultivars et les espèces sauvages de tomate et nous identifions les facteurs qui peuvent influencer des différences de taille des fruits. **Conclusion.** Bien que cela soit difficile, nous montrons qu’il est possible de produire des fruits présentant un rendement horticole élevé et nous utilisons la lignée sélectionnée « Solara » comme exemple. En outre, nous suggérons des pistes de recherches supplémentaires pour comprendre la régulation et le contrôle du contenu en glucides des fruits.

États-Unis / *Solanum lycopersicum* / fruits / sucres / métabolisme des glucides / teneur en glucides

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RESUMEN ESPAÑOL, p. 64

1. Introduction

The tomato is one of the most popular fruits, with global production estimated at ~1.26 Mt per year¹. Tomato fruits are an excellent source of vitamins A, C, E and lycopene, which collectively may lower the risk and occurrence of some cancers [1–3] and heart disease [4–6]. Both fresh and processed tomatoes are widely used in many traditional and modern dishes and the zeal for a ‘perfect-tasting’ tomato has spurred socio-political advocacy for local, sustainable, organic production of heirloom varieties [7]. One key component of fruit quality is total soluble solids (TSS). Although TSS of ripe tomato fruit is a measurement of several chemicals, it is a convenient proxy for sugar content [8]. Higher TSS positively influences consumer fruit likeability and reduces costs associated with processing tomatoes [8].

The problem is that fruit are bred for optimal postharvest handling [8] but, even when trying to genetically select for fruit with higher total soluble solids, a loss of yield occurs [9]. Exacerbating this problem is that commercial harvesting is usually done before the fruit reaches full-ripe and this cuts off the sugar supply from the fruit, leading to a less than favourable content [8]. One solution to this problem is to develop higher

TSS ‘value-added’ fruit to offset any loss of yield and to minimise the severity of the effect on sugars by premature harvesting. In our review we will look at the biochemical factors that help to determine the potential for high sugar accumulation.

2. Tomato fruit development

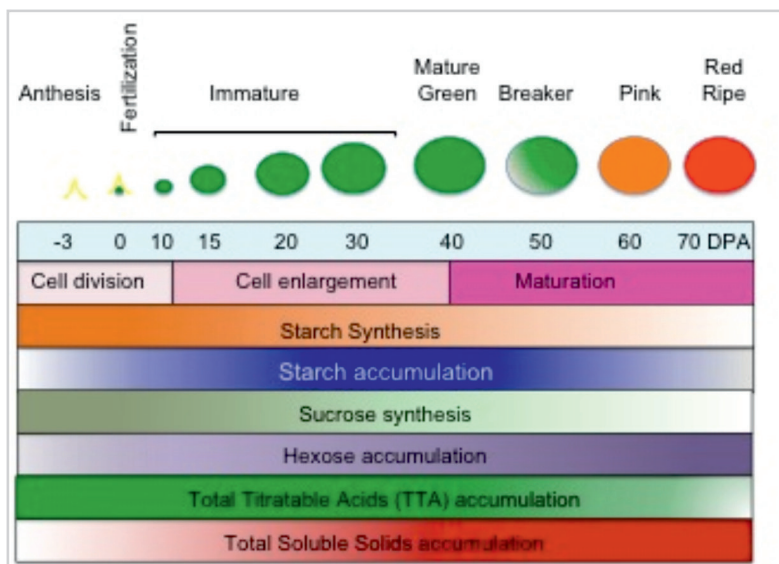
Fruit development from anthesis to full maturation is regulated by changes in endogenous and external environmental signals whose perception is relayed by hormonal and sugar signalling [10–13]. There are five recognisable phases [14]: anthesis, fertilisation, cell division, cell expansion and ripening, with some overlap between stages. Cell division occurs in the newly-formed fruit for 7–10 days [15], or in large-fruited cultivars for 20 days [16], after which the final fruit cell number is set. The cells then expand from 10–40 days post-anthesis (DPA) due to the vacuolar storage of photosynthate and water, leading to a more than ten-fold increase in fruit size [14, 17]. The final stage is ripening, in which the fruit undergoes several metabolic transformations brought on by climacteric ethylene, including the rapid import and accumulation of sugars, degradation of starch and synthesis of lycopene and carotenoids, the degradation of chlorophyll and the softening of the cell wall [18].

Tomato yield relies partly on cell division and partly on cell enlargement, events that occur in the green developing fruit, while fruit quality parameters are determined during ripening, especially after the climacteric period (*figure 1*). Early events influencing cell number and hence yield include [14] endoreduplication [19, 20], seed number [21] and hormone production, especially in seeds [17, 22–25]. The number of cells can then subsequently influence sink strength, *i.e.*, import of photosynthate from the source [26].

3. Carbohydrate metabolism in developing fruit

The carbon economy of the fruit changes as the organ matures. Sucrose is the major

Figure 1. Changes in fruit physiology and carbohydrate accumulation from anthesis to full maturity. Tomato classification as Immature, Mature Green, Breaker, Pink and Red Ripe are based on USDA Standards [95]. DPA are approximate and based primarily on a composite of data from *S. lycopersicum* L. cv. Moneymaker as described in Kortsee *et al.* [58], Luengwilai and Beckles [30], and Carrari *et al.* [105], and from other cultivars as described in Gillapsy *et al.* [14] and Schaffer and Petreikov [106]. Note starch synthesis is distinct from accumulation.



¹ FAOSTAT, FAO, 2006.

photoassimilate translocated to the fruit. The movement of this photoassimilate from source to sink occurs in the phloem, and is in response to a pressure gradient developed from differences in the osmotic potential at the site of phloem loading in the source, and at the site of unloading at the sink. Sucrose is transported to fruit cells via the symplast (through the plasmodesmata) or it may be metabolised in the apoplast by a cell wall invertase and the resulting hexoses imported via hexose transporters on the plasma membrane (*figure 2*). For several years the accepted view was that symplastic loading of sucrose predominated in young fruit while apoplastic loading of hexose occurred principally during ripening [27]; however, this paradigm has been questioned recently and the possibility that apoplastic loading may occur throughout fruit development has been suggested [25]. Sucrose in the cytoplasm is metabolised into UDPglucose and fructose by sucrose synthase (Susy) or into fructose and glucose by the neutral cytoplasmic invertase (*figure 2*). The activity of Susy, along with the hexokinases, may mobilise carbon from sucrose for the hexose phosphate pool, while sucrose metabolised by invertases may be destined for storage in the vacuole [27, 28], requiring active transport into that organelle [29]. Biochemical and molecular evidence suggests that both Susy and invertase activities are determinants of fruit sink strength [25].

In green fruit, hexose phosphates are mostly used for the synthesis of starch, which occurs rapidly from anthesis until 13 days post-anthesis (DPA) [30, 31] (*figures 1, 2*). This period also coincides with high levels of mitotic activity and the determination of the final cell number in the fruit [14, 32]. Fixing these newly imported sugars as starch may steepen the sugar gradient to the fruit and aid continued sugar import [30, 33–35]. On a per fruit basis, starch reaches maximal accumulation at ~40 DPA and is thereafter degraded in concert with ripening [30].

The starch and sucrose pools are turned over in tomato fruit. Sucrose re-synthesis may occur via Sucrose Phosphate Synthase (SPS) and Susy, and the activity of these enzymes remains relatively high during fruit

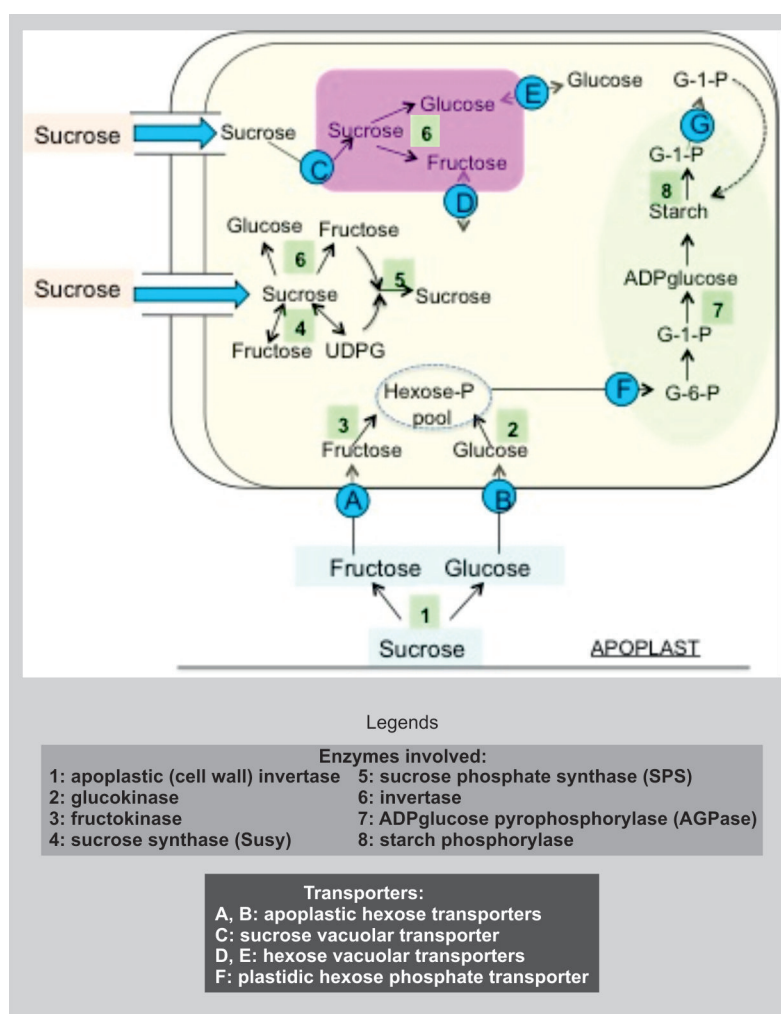


Figure 2.

Carbohydrate metabolism in developing tomato fruit. Sucrose may be imported directly via the symplast or may be inverted in the apoplast to hexoses which are then imported into the cell. Both sucrose and hexoses may be stored in the vacuole. The flux of sucrose to starch occurs early in fruit development from anthesis to ~20–25 days post-anthesis (DPA). Here, sucrose metabolism *via* sucrose synthase and hexokinases dominates. Hexose phosphate intermediates are then imported into the plastid for the synthesis of starch. As the fruit ripens, Susy and hexokinase activity declines relative to invertase and the apoplastic import of hexose becomes more significant with storage of sugars in the vacuole. Starch biosynthesis is minimal and active degradation of the starch occurs, which may add to the sugar content available for storage.

development [28, 36]. The enzyme isoforms involved in the disassembly of starch during turnover are not known [27, 37]. Enzymes capable of degrading starch via amylolytic routes have been detected in the plastids of

Table I.
Sources of high soluble solids traits in wild tomato species.

Wild species of <i>Solanum</i>	Fruit colour	Locus	Primary storage sugar(s)	Mechanism reported ¹	Reference
<i>S. cheesmanii</i>	yellow	–	fructose, glucose	Increased sugar import during ripening	Balibrea <i>et al.</i> [45]
<i>S. pimpinellifolium</i>	red	–	fructose, glucose	Increased invertase activity	Husain <i>et al.</i> [52, 53]
<i>S. pennellii</i>	green	<i>Lin5</i>	fructose, glucose	Apoplastic invertase with altered activity	Fridman <i>et al.</i> [50]
<i>S. chmielewskii</i>	green	<i>sucr</i>	sucrose	Reduced fruit acid invertase activity	Chetelat <i>et al.</i> [96]
<i>S. habrochaites</i>	green	<i>Agp-L1</i>	sucrose	<i>Agp</i> ² and increased fruit starch	Petreikov <i>et al.</i> [33]
<i>S. habrochaites</i>	green	<i>fgr, frk</i>	higher fructose:glucose	Epistatic interaction between <i>frk</i> ³ and <i>fgr</i>	Levin <i>et al.</i> [97]
<i>S. neorickii</i>	green	–	sucrose	Unknown	Schauer <i>et al.</i> [56]
<i>S. peruvianum</i>	green	–	sucrose	Higher sucrose import during ripening	Stommel [49]

¹ This does not imply that the biochemical difference is the sole cause for changes in total soluble solids.

² *Agp* is ADPglucose pyrophosphorylase.

³ *Frk* is fructokinase.

developing tomato fruit as early as 10 DPA, but they have not yet been shown to be directly involved in starch breakdown [38, 39]. Substrate cycles of sucrose and starch may provide metabolic flexibility and help to maintain the fruit as a carbon sink [30].

4. Carbohydrate pathways in wild tomato fruit

It is perhaps ironic that one of the traits most sought after during the domestication of tomato was increased yield [40], which coincidentally led to lower fruit TSS [21]. However, genetic, molecular and biochemical characterisation of wild tomato species with high fruit TSS (10–15% compared with 4–6% in cultivars) have vastly improved our understanding of carbohydrate metabolism in tomato, and can be exploited in breeding programmes [41]. Even among landraces of *Solanum lycopersicum* L. there is a great deal of underutilised potential for new sources of high TSS [42–44].

Differences exist in the pathway steps (figure 2) that partially explain the distinct sugar profiles of wild and modern tomatoes

(table I). No single mechanism universally explains increased TSS across all of the species examined. However, in addition to metabolic alterations, increased import of sugars, especially during the later stages of fruit development, has been identified in *S. cheesmanii* [45], *S. chmielewskii* [35, 46], *S. pennellii* [47], *S. habrochaites* [48], *S. peruvianum* [49] and *S. pimpinellifolium* as a contributory factor [45]. And although some “key” enzymes vary several-fold in activity between cultivars and the wild relatives, there may also be subtle but widespread variation in many fruit enzyme activities throughout development [28].

Two biochemical modifications related to differences in sugar import during fruit development in wild tomato species have been identified; changes in invertase activity and changes in starch accumulation. The magnitude and direction of the changes vary from species to species.

Solanum chmielewskii, *S. peruvianum*, *S. neorickii* and *S. habrochaites* are sucrose-storers. All except *S. neorickii* (where it has not been studied) contain invertases less adept at converting sucrose into hexoses, which leads to high accumulation of the former during late fruit development [25, 35,

46, 50]. Rapid rates of sucrose recycling may also accelerate sucrose import in *S. habrochaites* [27]. Miron *et al.* found, in addition to lower invertase, higher activity of Susy and SPS. They proposed that this could amplify the sucrose gradient to fruit; sucrose would be metabolised by Susy in the cytosol with subsequent re-synthesis by SPS and import into the vacuole at greater rates than in the cultivar [48]. This is an attractive hypothesis and comports well with the idea that sucrose is turned over in fruit and with the higher SPS activity found in another sucrose-storer, *S. peruvianum*. However, in *S. chmielewskii* SPS and Susy activities similar to domesticated tomatoes were measured [51], suggesting that higher sucrose cycling may not occur in all sucrose-storers. There are no reports of these enzyme activities in *S. neorickii*.

In contrast to the sucrose-accumulating species, *S. cheesmanii* [45], *S. pennellii* [47, 50] and *S. pimpinellifolium* [52] store large amounts of glucose and fructose that may be conditioned by higher invertase activity compared with the cultivars. *Solanum pennellii* has been extensively studied. High apoplastic invertase activity was found in the columella which increased during ripening. This activity would magnify the sucrose gradient between the phloem and fruit parenchymal cells by the rapid inversion of sucrose to hexose [52, 53]. A similar mechanism may operate in *S. pimpinellifolium* in the apoplast to aid sink strength, but there must be other factors contributing to high TSS in this species. About 90% of the invertase activity in this species is vacuolar, not apoplastic, and vacuolar activity correlated with the linear accumulation of hexose during ripening [52, 53]. The situation is even less clear for *S. cheesmanii*. Only the cytosolic invertase activity was higher, and slightly so, when compared with the cultivar, and only at 20 DPA when sugar import for storage is minimal [45]. Also of note is that although *S. habrochaites* stores sucrose, it has a higher ratio of fructose to glucose. This is due to the presence of the *frg* allele and its epistatic interaction with fructokinase [54, 55]. This is desirable because fructose, along with sucrose, is sweeter than glucose [8], and attempts to engineer high

fructose by changing fructokinase activity have been unsuccessful.

Many high-TSS wild tomatoes also show alterations in starch metabolism. *Solanum chmielewskii* [56], *S. pennellii* [28, 47] and *S. habrochaites* (formerly *Lycopersicon hirsutum*) [57] accumulate more starch, while *S. peruvianum* accumulates less starch at some stages [58] compared with *S. lycopersicum*. The mechanism by which altered starch accumulates in some wild species is not widely known except for *S. habrochaites*. This species harbours a modified allele of the large subunit of ADPglucose pyrophosphorylase (AGPase; *figure 2*) which is a key enzyme of starch biosynthesis. The *S. habrochaites* AGPase remains active for an extended period, leading to higher levels of starch biosynthesis in the fruit [33, 58]. Petreikov *et al.* proposed that this modification simultaneously enhances sink capacity, thereby leading to higher sugar accumulation, and the 'extra' starch adds more sugars to the final reserves in ripe fruit [33, 59]. It may not be surprising that some 'sucrose-storers' would synthesise more starch since it has been posited that the amount of starch synthesised in fruit is driven by sucrose content [27, 37]. Schauer *et al.* also suggested that since many of these fruit remain green, photosynthetic activity may also contribute to starch [56].

Finally, a major caveat in making cross-comparisons of fruit metabolism between studies as we have done is the potential for drawing inaccurate conclusions. For example, *Solanum chmielewskii* was shown to accumulate more starch than the cultivar [56] but there are two reports that this species accumulates less starch [35, 45]. And, peculiarly, *Solanum cheesmanii* does not have the large increase in invertase activity expected of high-TSS hexose-storers. These observed disparities could be due to a number of reasons: (i) variation in the fruit tissues sampled, *i.e.*, pericarp *vs.* columella *vs.* whole fruit. Baxter *et al.* found higher uptake of sugars in the columella but not in the pericarp of *S. pennellii*, pointing to the importance of enzyme tissue specificity [47]. Therefore, the modest levels of invertase activity found in *S. cheesmanii* may be a result of sampling only the pericarp and not

the columella; (ii) variation in fruit maturation at the time of sampling due to developmental shifts between wild tomatoes and cultivars [28], and (iii) differences in the accessions sampled. For example, some accessions of *S. pimpinellifolium* have invertase activity similar to cultivars [52].

5. Fruit size and yield in wild tomato fruit

Wild tomato species that produce fruit with high TSS content are low-yielding, while the inverse is true for cultivars. This link will be important to break if the tomato industry wishes to meet its goal of increasing TSS in large-fruited cultivars. Three mechanisms can be put forth to explain this. First, in large-fruited cultivars there could be “dilution effects” [45, 60]. Hexoses have a higher osmolarity compared with sucrose which would lead to a greater influx of water to the cells and, consequently, larger cell volumes and fruit sizes [46]. Second, sequence polymorphisms in the *fw2.2* allele can account for 30% of the difference in fruit size between wild species and cultivars by altering cell division in the pre-anthesis ovary [40]. Third, the relative proportion of hexose to sucrose in fruit may differ between wild and cultivated types during cell division, directly affecting this process. In *Arabidopsis* and legume seeds a high hexose-to-sucrose ratio at cell division stimulates mitotic activity, leading to more cells and a larger organ [61–63]. If this phenomenon is universal then it may be part of the reason for differences in fruit size among tomato species. When the fruit sugar profiles of *S. lycopersicum* were compared with those of the small-fruited *S. chmielewskii* [46], *S. habrochaites* and *S. peruvianum* [58], the hexose-to-sucrose ratio during cell division was significantly higher in the former, suggesting that this could contribute to the differences in fruit size between these two species. This thesis requires further testing on a broader spectrum of tomato species. Also interesting would be to establish if *fw2.2*, which is regulated by sugars [10],

shows a differential response to hexose *vs.* sucrose.

6. Developing tomato lines with high total soluble solids content and good yield – Solara: A case study

Fruit with modest increases in TSS but with no yield penalty have been produced through crosses involving *S. chmielewskii* [64] and *S. pennellii* [65] (TSS increases of 10–12% and 6%, respectively) and, more recently, yield increases were introduced into a tomato variety with no concomitant change in TSS [66]. However, engineering high horticultural yield is difficult due to the constraints imposed by source-sink relations. We studied ‘Solara’, a breeding line with fruit that are twenty-fold larger than those of *S. pimpinellifolium* L., from which it was derived, and yet are high in TSS (9–11%), and that appears to be an exception to this general rule [67].

‘Solara’ was derived by crossing a Bulgarian cultivar (*Solanum lycopersicum* L.) and *S. pimpinellifolium* L., followed by 10 years of selections among the segregating population. In the experiment described (*table II*), ‘Solara’ had a 30% higher horticultural yield compared with *Solanum lycopersicum* L. cv. Moneymaker. The fruit have high TSS from 9–11% (*table II*), lower than that of cherry tomato hybrids (10–15%) and the *S. pimpinellifolium* parent (12%; [68, 69]), but higher than most table or processing varieties (4–6%; [35]). In addition, summer field-grown ‘Solara’ fruits’ TSS approaches 10–13% (L. Stamova, unpubl. data) in multiple tests. ‘Solara’ fruits’ Total Sweetness Index (TSI) of 6.7 ± 0.2 (*table II*) is higher than the normal range of 3.8 to 5.0 reported for fresh market tomato varieties, indicating an exceptionally sweet fruit [70]. The fruit has 2–3 locules and a low proportion of gelatinous tissue which may further contribute to its high [TSS / TA] ratio [71]. When grown under suboptimal conditions (Winter, *table II*), ‘Solara’ fruits’ TSS were unchanged (9%), suggesting a strong genetic basis for this trait.

Table II.

Performance of 'Solara' grown in two different environments, in a glasshouse in summer 2007 (Davis, CA, USA), and in a plastic greenhouse in winter 2006 (Winters, CA, USA). Each measurement is the mean $\pm$ standard error of the mean from at least 6 plants per line. Plants were pruned so that only nine trusses were produced per plant in the summer and five in the winter. Plants of the cv. 'Money-maker' were grown in a randomised block design with 'Solara' plants in summer 2007. The mean temperature was 18 °C at night and 25 °C during the day in summer 2007, and 4 °C at night and 15 °C during the day in the winter 2006. Yield was calculated as described by Luengwilai *et al.* [67]. All measurements were made at 13 weeks after germination except TSS and TA, which were measured on ripe fruit. All biochemical and postharvest methods were carried out as described previously [67, 100].

Parameter	Number of fruit per truss	Number of fruit per plant	Fruit fresh weight (g)	Fruit circumference (cm)	Yield (kg·truss ⁻¹)	TSS (°Brix)	TSS × Yield (°Brix·kg·truss ⁻¹)	TA (g citric acid·100g ⁻¹)	[TSS / TA] ratio	TSI (relative units)
Solara										
Winter 2006	9.0 $\pm$ 0.5	32.0 $\pm$ 3.0	19.2 $\pm$ 0.6	10.5 $\pm$ 0.2	0.12 $\pm$ 0.008	8.9 $\pm$ 0.9	1.1 $\pm$ 0.1	0.6 $\pm$ 0.0	14.0 $\pm$ 0.9	2.9 $\pm$ 0.08
Summer 2007	12.1 $\pm$ 0.3	42.5 $\pm$ 5.1	30.2 $\pm$ 0.8	11.6 $\pm$ 0.1	0.13 $\pm$ 0.01	9.1 $\pm$ 0.1	1.2 $\pm$ 0.01	0.3 $\pm$ 0.0	32.0 $\pm$ 1.2	6.7 $\pm$ 0.2
<i>P</i> -value	0.0003*	0.106	< 0.0001*	0.0006*	0.404	0.829	0.294	0.0012*	< 0.0001*	< 0.000
Money-maker										
Summer 2007	3.90 $\pm$ 0.48	15.40 $\pm$ 1.19	38.01 $\pm$ 2.16	14.02 $\pm$ 0.36	0.15 $\pm$ 0.02	<0.0001*	0.87 $\pm$ 0.03	0.64 $\pm$ 0.001	9.11 $\pm$ 0.5	5.13 $\pm$ 0.3
<i>P</i> -value	< 0.0001*	0.0004*	0.0069*	< 0.0001*	0.3921	5.83 $\pm$ 0.37	< 0.0001*	< 0.0001*	< 0.0001*	0.0014*

*: Significant at $P < 0.05$.

Sugars were measured by High Performance Liquid Chromatography.

TSS: Total Soluble Solids content measured by refractometry.

TSI: Total Sweetness Index; TSI was calculated according to Clarke [98], as TSI = (1 sucrose + 0.76 glucose + 1.50 fructose) using sugar accumulated as a percentage of fresh weight.

TA: titratable acids measured as described by Luengwilai *et al.* [99].

Table III.

Role of carbohydrate genes in tomato fruit derived from transgenic manipulation.

Enzyme	Manipulation ¹	Fruit phenotype	Reference
Apoplastic invertase	<i>RNAi</i> ² , <i>CaMV</i> ³	↑sucrose, ↓ hexose, reduced fertility, fruit set and fruit size	Zanor <i>et al.</i> [25]
Acid invertase ⁴	Antisense ² , <i>CaMV</i>	↑ sucrose, ↓ hexoses and fruit size	Ohyama <i>et al.</i> [24]; Klann <i>et al.</i> [17]
Sucrose synthase 1 ⁵	Antisense, <i>CaMV</i>	↓ sucrose unloading at 7 days after anthesis, ↓ fruit set	D'Aoust <i>et al.</i> [101]
Sucrose synthase 1 ⁵	Antisense, <i>2A11</i> ⁶	No detectable change in starch or sucrose levels	Chengappa <i>et al.</i> [102]
Hexokinase 1	Ectopic <i>AtHK</i> ⁷ , <i>CaMV</i>	↓ fruit size, seed dry weight, starch content, total soluble solids at breaker stage and red ripe stage	Menu <i>et al.</i> [22]
Fructokinase 1	Antisense, <i>CaMV</i>	Delayed flowering, ↑ fruit sucrose	Odanaka <i>et al.</i> [23]
Fructokinase 2	Antisense, <i>CaMV</i>	↓ seed number, flower and fruit set, ↑ fruit sucrose	Odanaka <i>et al.</i> [23]
Vacuolar H ⁺ -ATPase	Antisense, <i>2A11</i>	↓ fruit weight, seed number, ↑ sucrose	Amemiya <i>et al.</i> [103]

¹ Transgenic manipulation and gene promoter used.² *RNAi* and antisense methods are used to repress gene expression.³ Cauliflower Mosaic Virus 35S gene promoter.⁴ Both apoplastic and vacuolar acid invertase.⁵ Isoform designation from Goren *et al.* [104].⁶ Fruit-specific gene from tomato ("*2A11*") promoter.⁷ Ectopic overexpression of *Arabidopsis* hexokinase 1.

Biochemical and physiological analysis of 'Solara' points to high import of sugars (primarily glucose and fructose) over a longer period compared with Moneymaker [67]. Sucrose is synthesised at high rates but accumulation, while higher than in Moneymaker, does not match synthesis, indicating higher rates of cycling [67]. Starch turnover at the red ripe phase was detected in 'Solara' but not in Moneymaker by ¹⁴C-glucose pulse-chase experiments (unpubl. data).

7. Understanding the role of fruit metabolism in determining fruit sugar content

There are several basic questions regarding tomato fruit carbohydrate metabolism that remain unanswered. Transgenic alterations, and repression or overexpression of various genes have provided some valuable infor-

mation on the roles of various enzymes in fruit metabolism (*table III*; [72]). We agree with others that knowledge of metabolic fluxes and enzyme activity will be important in putting the puzzle together, and that this cannot be overstated [28, 73, 74]. There are areas 'ripe' for investigation into tomato fruit carbon fluxes that we still need to understand.

1. Susy, SPS and AGPase [27] are highly regulated enzymes that may be constrained by regulatory loops [73, 75]. Expression of enzymes modified by site-directed mutagenesis may promote increased flux through the pathway at these points. For instance, changes in the activation state of AGPase lead to more starch and higher TSS (see point 7).

2. Many fruit enzymes involved in carbohydrate metabolism have multiple isoforms, each with unique kinetic properties and restricted spatio-temporal occurrence (*e.g.*, invertases, Susy, fructokinase). Sequential and combinatorial "knocking out" of the

various isoforms as demonstrated by Barratt *et al.* [76] would contribute to our basic knowledge of their unique and shared contributions to total enzymatic activity.

3. Membrane transporters are the gateways for the movement of metabolites and compounds between compartments [77] and they can have all-encompassing effects on fluxes. But the roles of several of these transporters remain under-studied in tomato fruit (figure 2). There is now evidence for the regulation of plant sugar transporters by endogenous sugar levels via kinases [78] which adds an interesting layer of complexity to delineating their role in carbohydrate accumulation in the fruit.

4. The subcellular concentrations of metabolites and sugars should be estimated in order to determine potential changes in enzyme activity during development [79]. For example, Susy and fructokinase are inhibited by physiological levels of fructose [80]. Knowing the concentration of fructose in the cytosol may refine our view of how this enzyme works during fruit development.

5. Sugar signalling and sensing by invertase, hexokinases and as yet unidentified proteins should also be investigated. Invertases convert sucrose into hexoses, which in turn are used for carbon, energy and as signalling molecules and, by inference, are implicated in regulating all aspects of growth and development, including carbon partitioning to sinks [76, 81, 82]. The expression of each fruit invertase isoform – apoplastic, cytoplasmic and vacuolar – should be repressed individually and in combination. Zhanor *et al.* used RNAi to silence *LIN5*, the apoplastic invertase in *S. lycopersicum*. Altered *LIN5* is the basis for high TSS in *S. pennellii*. In so doing, they uncovered a web of interconnections between sugar content, and fruit development, fertility and importantly regulation of hormonal levels [25]. Hexokinases are sugar sensors and are central to sugar signalling in plants [83, 84]. Overexpression of *Arabidopsis HK1* was performed using a constitutive promoter, which led to many developmental effects [22, 85, 86]. The consequence of overexpressing the native HK by using a fruit-specific promoter should also be pursued [17, 24].

6. Little is known about the regulation of substrate cycles of sucrose and starch in developing tomato fruit. Starch and sucrose levels are known to vary across cultivars and species and it seems reasonable to expect that these cycles may be regulated differently depending on genetic background. Transgenic manipulation of the degradation reaction may be one way to increase carbohydrate reserves [87].

7. Starch metabolism appears to help determine yield and/or TSS in some tomato cultivars and species. As previously mentioned, higher TSS in some wild tomatoes is associated with altered starch metabolism but now there is evidence to support this in cultivars, although changes in starch are not a universal path to high TSS. Dinar and Stevens made a link between young fruit starch content and fruit TSS and the results of other correlative studies support this [88, 89].

There is now evidence that starch plays a more direct role in determining TSS and yield. Two studies of transgenic lines whereby AGPase was overexpressed and suppressed, respectively, provided some clues. In the first study, an unregulated bacterial AGPase was transformed into tomato and this purportedly led to higher starch and increased TSS [90], but this report is controversial [87]. In another study, repression of the tomato AGPase activity by 90% apparently reduced starch to 25% of the levels found in wild-type and led to delayed flowering and lower yield. Although the results were tantalising in suggesting a relationship between starch and sink strength, as the authors noted, they could not rule out that the changes were due to somaclonal variation [91].

Second, it has long been observed that tomatoes subject to salt stress accumulate higher levels of starch in green fruit, and have elevated TSS in ripe fruit [45, 92, 93]. Initially, this was explained as a function of dilution effects, *i.e.*, the high electrical conductivity meant the phloem supply to the fruit was more concentrated. Now, Yin *et al.* have provided direct proof that starch has a pivotal role since changes in salinity and osmotica altered AGPase at the transcriptional and posttranscriptional level, respectively, increasing green fruit starch

biosynthesis [93]. As explained previously, partitioning of carbon to starch may increase sink strength in green fruit and then when it is degraded during ripening adds to the pool of sugars imported from the phloem [33, 34, 59].

Third, perhaps the best evidence for a role of starch in determining TSS in cultivars was found in transgenic tomatoes perturbed in malate content [94]. This set up cellular redox changes which altered the activation state of AGPase and, in turn, starch accumulation that was directly linked to modulation of fruit TSS [94]. Collectively these data point to an important role for starch metabolism in determining fruit carbon fluxes. There appear to be great differences in starch metabolism among cultivars [89] and there is evidence that granule degradation may be highly regulated [30]. It is intriguing, the possibility that flux in and out of starch could represent a core control point for tomato fruit metabolism.

8. Conclusion

Fruit traits are ultimately defined by a culmination of molecular events. With genomic resources readily accessible for tomato, comparative cross-species analyses of DNA, RNA, protein and metabolites within the *Solanaceae* is possible. Sugars along with hormones are powerful regulators of organ growth, development and metabolism, and these factors are often intertwined to determine the sugar-fruit size dynamic. However, as we are finding out, enzyme activities and biochemical flux analysis of high-TSS tomato species may still be indispensable in advancing our knowledge of the processes underlying fruit sugar accumulation.

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Factores bioquímicos que contribuyen al contenido de azúcar de los frutos de tomate: un repaso.

Resumen — Introducción. Los consumidores e industriales aprecian los tomates con un fuerte índice de azúcares, pero la mayoría de las prácticas relativas al cultivo y de mejora tienen un impacto negativo sobre este rasgo característico. Las especies de tomate salvaje pueden acumular 2 ó 3 veces más azúcares en el fruto que los cultivares, y resultan ser apreciadas como fuente de *loci* de alto contenido en azúcares para aumentar la base genética de los cultivares actualmente producidos y como material de investigación para comprender dicho rasgo característico. **Síntesis.** A pesar de que los acercamientos genómicos punteros nos hayan enseñado mucho sobre el fenotipo de los frutos, sigue siendo importante evaluar la actividad de las encimas de los frutos, así como los flujos metabólicos en líneas que presenten situaciones contrastadas de acumulación de azúcares en los frutos. Dichas funciones metabólicas son las que más se acercan al rasgo característico del contenido de azúcares en el fruto maduro. En esta síntesis, nos centramos en las vías bioquímicas, particularmente en la biosíntesis del almidón, que puede influenciar los azúcares en el fruto del tomate. Intentamos, en la medida de lo posible, situar esta información en un contexto fisiológico, ya que, conjuntamente, influyen el rendimiento. Comparamos y contrastamos el metabolismo de los azúcares en los cultivares y en las especies salvajes de tomate, e identificamos los factores que pueden influenciar las diferencias en el tamaño de los frutos. **Conclusión.** A pesar de la dificultad, ilustramos la posibilidad de producir frutos que presenten un elevado rendimiento y utilizamos la línea seleccionada « Solara » como ejemplo. Además, sugerimos vías de investigación suplementarias para comprender la regulación y el control del contenido de glúcidos de los frutos.

EUA / *Solanum lycopersicum* / frutas / azúcares / metabolismo de carbohidratos / contenido de carbohidratos